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Short-term effects of dietary supplementation with amino acids in dogs with proteinuric chronic kidney disease

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Abstract: This retrospective study investigated the impact of amino acid supplementation on body weight, serum albumin, creatinine and urea concentrations, and urine protein-to-creatinine (UPC) ratio in proteinuric dogs with chronic kidney disease (CKD). Forty-six client-owned azotemic dogs with spontaneous proteinuric CKD already on a renal diet and in therapy with enalapril were included. After approximately 1 month of treatment (baseline), 29 dogs received oral amino acid supplementation daily (group A) and 17 dogs did not (group B). The parameters under investigation were determined at baseline and after 4 to 8 weeks in both groups. Compared to baseline, body weight and serum albumin increased ($P < 0.01$, $P < 0.05$, respectively) at follow-up in group A, but did not change in group B. Serum creatinine concentration did not change in both groups; urea concentration ($P < 0.05$) and UPC ratio ($P < 0.01$) decreased in group B, but not in group A. Supplementation with amino acids increased body weight and serum albumin concentration in these dogs but it might have prevented a decrease in proteinuria and urea concentration.

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**SHORT-TERM EFFECTS OF DIETARY SUPPLEMENTATION WITH AMINO ACIDS IN
DOGS WITH PROTEINURIC CHRONIC KIDNEY DISEASE**

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22 Abstract

23 As malnourishment in humans with chronic kidney disease (CKD) is associated with increased
 24 morbidity and mortality, oral supplementation with amino acids is proposed. In dogs, malnutrition
 25 may be associated with CKD and increases the rate of renal-related complication and mortality.
 26 Aim of this retrospective study was to verify the impact of amino acid supplementation on body
 27 weight, serum albumin, creatinine and urea concentrations, and urine protein-to-creatinine (UPC)
 28 ratio in proteinuric CKD dogs. Forty-six client-owned azotemic dogs with spontaneous proteinuric
 29 CKD already on a renal diet and in therapy with enalapril were included. After approximately one
 30 month of treatment (baseline), 29 dogs received oral amino acid supplementation daily (group A),
 31 whereas 17 dogs did not (group B). The amount of amino acids was calculated as follows: body
 32 weight (kg) \times [UPC ratio] \times 20 = X , where X are the milligrams of amino acids to be administered
 33 daily. Body weight, serum albumin, creatinine and urea concentrations, as well as the UPC ratio
 34 were determined at baseline and after 4-8 weeks in both groups. Compared to baseline, body weight
 35 increased ($P<0.01$) at follow-up in group A, while it did not change in group B. Serum albumin
 36 concentration increased ($P<0.05$) in group A and it did not change in group B. Serum creatinine
 37 concentration did not vary in both groups; urea concentration ($P<0.05$) and UPC ratio ($P<0.01$)
 38 decreased in group B, but not in group A. Although supplementation with amino acids increased
 39 body weight and serum albumin concentration in dogs, maintaining stable serum creatinine
 40 concentration, it might have **prevented a decrease of proteinuria and of urea**. Amino acid
 41 supplementation may be considered in the treatment of proteinuric dogs, if hypoalbuminemia is
 42 severe and antiproteinuric therapy is not effective.

43

44 **Abbreviations:** amino acids, AA; angiotensin converting enzyme inhibitors, ACEI; borderline
 45 proteinuria, BP; body weight, BW; chronic kidney disease, CKD; dry matter, DM; International
 46 Renal Interest Society, IRIS; non-proteinuric, NP; proteinuric, P; urine protein-to-creatinine, UPC.

47

48 **Introduction**

49 In dogs, proteinuria is often associated with chronic kidney disease (CKD) and studies in this
50 species led to hypothesize that it may promote the progression of renal damage, as it happens in
51 humans (1-8). In endemic areas for vector-borne diseases, such as leishmaniosis, the prevalence of
52 dogs with proteinuria, azotemia, or both, has been reported to be up to 50% (8,9). Among dogs at
53 risk for developing proteinuric nephropathy, other than those living or having lived in endemic
54 areas, there are also breeds that are genetically predisposed to proteinuric CKD (3,4,6,8). Early
55 identification and treatment of proteinuria appears to be crucial in dogs, not only because of the
56 high prevalence, but also as its management slows the progression of renal disease, risk of uremic
57 crisis and renal-related death (1,3,4). Together with the treatment of the underlying disease, the
58 major cornerstones of therapy in proteinuric CKD dogs are angiotensin converting enzyme
59 inhibitors (ACEI), dietary intervention and **omega-3** fatty acids which proved to slow the
60 progression of renal disease, minimize clinical signs of uremia and, at least for the diet, to maintain
61 either an optimal body weight (BW) and body condition score (1-3,6,9-14). However, in some dogs,
62 anti-proteinuric therapy may not reduce proteinuria **despite diets lower in protein compared to**
63 **maintenance diets** are **administered concurrently** (2-4,8-11,13,14). Furthermore, in proteinuric
64 dogs renal diets may not adequately meet protein requirements, thus possibly leading to low BW,
65 hypoalbuminemia and malnutrition. A previously published study on a spontaneous model of
66 proteinuric nephropathy in dogs, showed that a low protein content diet (14% on dry matter basis),
67 similar to the renal diets commonly recommended in dogs with kidney disease, caused a significant
68 reduction in BW and plasma albumin concentration that were already noticeable at 4 weeks of
69 administration (8).

70 In humans with CKD, nutritional status is helpful to identify patients with increased risk of
71 morbidity and mortality; a significant association, indeed, was observed between decreased baseline
72 BW condition and subsequent risk of hospital admission (15-18). For these reasons, in

malnourished humans with CKD oral supplementation with amino acids (AA) or intradialytic AA administration have been proposed (15-19).

In CKD dogs with severe proteinuria, either low BW and hypoalbuminemia are frequent and they can be associated with increased morbidity and risk of mortality (20). Indeed, albumin hypercatabolism and its down-regulated synthesis can contribute to glomerular disease-associated hypoalbuminemia, possibly leading to marked hypoalbuminemia in CKD dogs with sub-nephrotic range proteinuria thus worsening the prognosis (21). Based on this premise, it seems plausible that the amount of proteins needed should be individually-tailored in dogs, depending on the stage of CKD and the entity of proteinuria (8). As in humans, also in dogs AA supplementation may represent the easiest means to correct an insufficient daily intake of proteins. Thus, the aim of this retrospective case-control study was to investigate the impact of an oral AA supplementation during a short period of time on BW, serum concentrations of albumin, creatinine and urea, and on the urine protein-to-creatinine (UPC) ratio in proteinuric CKD dogs treated with enalapril and fed a commercial renal diet (RD).

88 **Materials and Methods**

89 *Animals and inclusion criteria*

90 Medical records of proteinuric dogs in IRIS stages ≥ 2 (14) admitted in 2007 and 2008 at one of the
 91 authors' institutions (AZ, PDI) were reviewed. All the data available regarding clinical history,
 92 physical examination, BW, complete blood count, serum biochemical profile, urinalysis, UPC ratio,
 93 indirect blood pressure measurements, abdominal ultrasonographic findings, ongoing treatments
 94 and follow-up examinations were collected. Dogs without stable renal function were excluded;
 95 stable renal function was defined by serum creatinine concentration that did not increase or decrease
 96 by 20% or more within one month from initial determination (10). Dogs were considered to be
 97 proteinuric if the UPC ratio was above 0.5 (IRIS substage P) (14) in two urine samples collected at
 98 one-month interval; dogs that did not fulfil this criterion were excluded. Furthermore, to be included
 99 in the study, dogs had to receive enalapril (Enacard[®], Merial Italia spa, Milano, Italy) at 0.5 mg/kg
 100 (0.23 mg/lb), q 24 h, and a commercial renal diet (Hill's Prescription Diet Canine k/d[®], Hill's Pet
 101 Nutrition Inc., Topeka, KS or Royal Canin Renal Canine[®], Royal Canin SA, Aimargues, France);
 102 **the amount of diet was according to the suggestions of the companies and corrections were not**
 103 **performed if dogs received or not AA supplementation.**

104 From each record, information was collected to identify dogs that received or not oral AA
 105 supplementation (IT IS pet, ACME srl, Cavriago (RE), Italy; formulation available in table 1).
 106 Among dogs on AA supplementation, only those taking the daily amount (mg) of AA arbitrarily
 107 calculated using the following formula, $BW \text{ (kg)} \times [UPC \text{ ratio}] \times 20 = X$ (22), where X are the
 108 milligrams of amino acids to be administered daily were included. Based on the product indications,
 109 1 tablet provides approximately 675 mg of AA. Dogs that had received oral or intravenous AA
 110 supplementation within one month from time of admission were excluded. Finally, dogs were
 111 excluded if the diagnostic workup identified any inflammations or infections of the genitourinary
 112 tract (based on ultrasonography and urinalysis), a pre-renal cause of proteinuria (based on serum
 113 biochemistry), and if cardiac disease, neoplasia or endocrinopathies were diagnosed or suspected.

114 All dogs had been tested for leishmaniosis, ehrlichiosis and babesiosis, and were not included if an
115 active form of infection was either identified or suspected.

116

117 *Additional treatments and follow-up*

118 As a standard of care at the authors' institution, dogs classified as "severely hypertensive" (systolic
119 arterial pressure ≥ 180 mm Hg) accordingly to the IRIS staging system (14) were treated with oral
120 amlodipine at 0.1 to 0.5 mg/kg (0.05 to 0.23 mg/lb), q 24 h, in order to reduce systolic arterial
121 pressure to < 160 mm Hg (substage "normotensive" or "borderline hypertensive"). In addition, dogs
122 with severe hypoalbuminemia received oral acetylsalicylic acid at 2.0 mg/kg (0.91 mg/lb), q 24 h,
123 to prevent thrombosis. Based on the reference range of serum albumin (2.8 to 3.8 g/dL), dogs were
124 considered hypoalbuminemic if the albumin concentration was ≤ 2.7 g/dL; severe hypoalbuminemia
125 was arbitrarily defined as a value < 2.0 g/dL.

126 As stated above, dogs with proteinuric CKD were reassessed after one month to check if the renal
127 disease was stable; all throughout the manuscript this time-point will be called *baseline*. After
128 baseline, dogs were re-evaluated between 4-8 weeks.

129

130 *Blood sampling and assay*

131 During each examination, blood samples were collected in dogs fasted overnight, and serum was
132 obtained within 30 minutes, stored at 4 °C (39 °F) and analyzed within 24 hours. Results from
133 complete blood count and serum biochemical analysis, including albumin, total proteins, glucose,
134 bilirubin, cholesterol, amylase, alanine transferase, alkaline phosphatase, urea nitrogen, creatinine,
135 sodium, potassium, chloride and phosphate, were achieved with the same methods (BC-2800Vet,
136 MINDRAY, Mindray Co. Ltd., Shenzhen, China; Cobas Mira, Roche Diagnostic AG, Basel,
137 Switzerland) in all samples.

138

139 *Urine collection and urinalysis*

140 An ultrasound-guided cystocentesis was performed in all dogs using a 5 mL syringe connected to a
 141 23-gauge needle. All urine samples were placed in 10 mL, sterile, evacuated collection tubes, and
 142 analyzed by the same operator. **Urine samples** were examined within 60 minutes from collection if
 143 samples were stored at room temperature (approximately 20 °C [67.6 °F]), or within 4 hours if
 144 stored at 4 to 8 °C (39.2 to 46.4 °F). Urine sediment was obtained by centrifugation (10 minutes at
 145 900 × g) of 5 mL of urine, followed by removal of 4.5 mL of supernatant, and resuspension of the
 146 remaining 0.5 mL of urine. A sample of 12 µL of the resuspended urine was microscopically
 147 assessed. The supernatant was transferred into separate tubes and stored at –20 °C (–4 °F) to
 148 determine UPC ratio within 7 days. Red blood cells and white blood cells were expressed as mean
 149 number of cells/10 hpf (40 × magnification). Urine sediment with bacteriuria, and/or >5 red blood
 150 cells or white blood cells/hpf, was considered indicative of active inflammation and excluded from
 151 the UPC ratio evaluation (23).

152

153 *UPC ratio*

154 To calculate the UPC ratio, protein concentration (mg/dL) was measured with pyrogallol red, and
 155 creatinine (mg/dL) was measured using the Jaffé method on undiluted urine supernatant that was
 156 thawed before analysis. Analytes were measured in an automated spectrophotometer (Cobas Mira,
 157 Roche Diagnostic AG, Basel, Switzerland) in each case.

158

159 *Statistical analysis*

160 For data evaluation, BW, serum albumin, creatinine and urea concentrations, and the UPC ratio
 161 were retrieved from baseline and after 4-8 weeks in all dogs. Dogs that received the AA
 162 supplementation were included in the group A, those that did not receive the AA supplementation
 163 belonged to the group B (no placebo was provided). To verify whether population characteristics
 164 were similar in the two groups, baseline age, BW, serum albumin, creatinine and urea

165 concentrations, and UPC ratio were compared with unpaired t-test. Sex distribution, and frequency
166 and severity of hypoalbuminemia were compared between groups with chi-squared test or Fisher's
167 exact test.

168 To study the effect of AA supplementation, BW, serum albumin, creatinine and urea
169 concentrations, and UPC ratio at baseline and after 4-8 weeks were compared within each group
170 with paired t-test. Because severe hypoalbuminemia may be associated with morbidity and
171 mortality in dogs (3,20,21), the effect of AA supplementation was also explored in the subset of
172 cases with serum albumin concentration <2.0 g/dL by paired comparisons between baseline and 4-8
173 weeks for the above parameters. Normality of all data sets was investigated with Kolmogorov-
174 Smirnov test and non-normally distributed variables were log-transformed to achieve Gaussian
175 distribution prior to using parametric tests. Results are reported as mean \pm standard deviation or as
176 percentages. A $P < 0.05$ was considered statistically significant. Statistical analysis was performed
177 with commercial software (GraphPad Prism version 4.0, GraphPad Software Inc., La Jolla, CA).
178

179 **Results**

180 *Baseline*

181 Forty-six proteinuric CKD dogs in IRIS stages 2, 3 or 4 were included; 29 of them received AA
 182 supplementation (group A), while the remaining 17 did not (group B).

183 Age and BW of both groups are reported in table 2. In group A, 20 (69%) dogs were males (17
 184 intact and 3 castrated) and 9 (31%) were females (8 intact and 1 spayed). Regarding dog breeds, 7
 185 were Boxer, 2 of each were German Shepherd, Dogue de Bordeaux, Epagneul Breton or Italian
 186 Pointer, and one of each was Cocker Spaniel, Dachshund, Dalmatian, Doberman, Dogo Argentino,
 187 German Pointer, Golden Retriever, Jack Russel Terrier, Pitt Bull and Rottweiler; the remaining 4
 188 dogs were cross-breed. In group B, 11 (64.7%) dogs were intact males and 6 (35.3%) were females
 189 (5 intact and 1 spayed). Regarding dog breeds, 3 were Boxer, 2 were Great Dane, and one of each
 190 was American Staffordshire, Dalmatian, Dogo Argentino, Dogue de Bordeaux, English Setter,
 191 German Pointer, German Shepherd, Irish Wolfhound, Labrador and Pomeranian; the remaining 2
 192 dogs were cross-breed. Age, sex distribution and BW did not significantly differ between groups.

193 Serum concentration of creatinine, urea and albumin, as well as UPC ratio of either group are
 194 reported in table 2. In group A, 18 (62.1%) dogs were in IRIS stage 2, 9 (31.0%) in IRIS stage 3 and
 195 2 (6.9%) were in IRIS stage 4. In group B, 7 (41.2%) dogs were in IRIS stage 2, 5 (29.4%) in IRIS
 196 stage 3 and 5 (29.4%) in IRIS stage 4. Serum concentration of creatinine was significantly lower in
 197 dogs in group A ($P<0.05$), whereas albumin and urea, and the UPC ratio did not significantly differ
 198 between groups. In group A, 20 (69.0%) dogs had low albumin concentration, 11 of which showing
 199 severe hypoalbuminemia; in group B, 11 (64.7%) dogs had low albumin concentration, 4 of which
 200 showing severe hypoalbuminemia. The frequency of dogs with hypoalbuminemia or severe
 201 hypoalbuminemia was not significantly different between groups A and B.

202

203 *Weeks 4-8*

204 In group A at follow-up, BW increased in 16 (55.2%) dogs (**range**, from 0.5 to 4 kg), remained
 205 equal in 11 (37.9%) and decreased in 2 (6.9%); by arbitrarily considering BW as stable if increased
 206 or decreased by $\leq 2.5\%$, 14 (48.3%) of the 29 dogs had stable BW. The mean BW of dogs
 207 significantly increased by 6.2% (32 ± 15 kg; $P < 0.01$), compared to baseline. BW was available for
 208 10 out of 17 dogs of group B and was increased in 1 (10.0%) dog, equal in 5 (50.0%) and decreased
 209 in 4 (40.0%); BW was stable in 5 (50.0%) of the 10 dogs. The mean value did not differ from
 210 baseline (26 ± 12 kg; $P > 0.05$) (Figure 1).

211 In group A, serum albumin concentration increased in 19 (65.5%) dogs, was equal in 2 (6.9%) and
 212 decreased in 8 (27.6%); by arbitrarily considering albumin as stable if increased or decreased by
 213 $\leq 5\%$, 8 (27.5%) of the 29 dogs had stable albumin. The mean albumin concentration significantly
 214 increased by 0.2 g/dL (2.6 ± 0.7 g/dL; $P < 0.05$), compared to baseline. None of the 19 dogs with
 215 higher than baseline albumin had concentrations above the reference range. In group B, albumin
 216 concentration increased in 8 (47.1%) dogs, was equal in 2 (11.8%) and decreased in 7 (41.2%);
 217 albumin was stable in 4 (23.5%) of the 17 dogs. The mean value did not differ from baseline ($2.4 \pm$
 218 0.8 g/dL; $P > 0.05$) (Figure 2).

219 Serum concentration of creatinine in group A increased in 6 (20.7%) dogs and decreased in the
 220 remaining 23 (79.3%); by considering creatinine as stable if increased or decreased by $\leq 20\%$ (10),
 221 13 (44.8%) of the 29 dogs had stable creatinine. In group B, creatinine concentration increased in 4
 222 (23.5%) dogs, was equal in 2 (11.8%) and decreased in 11 (64.7%); creatinine was stable in 6
 223 (35.3%) of the 17 dogs. In both groups, mean serum concentration of creatinine measured at 4-8
 224 weeks did not statistically differ from baseline (group A: 2.0 ± 2.0 mg/dL; group B: 3.4 ± 3.3
 225 mg/dL; $P > 0.05$ for both).

226 In group A, serum concentration of urea increased in 9 (31.0%) dogs, was equal in 1 (3.4%) and
 227 decreased in 19 (65.5%); by arbitrarily considering urea as stable if increased or decreased by

228 $\leq 20\%$, 13 (44.8%) of the 29 dogs had stable urea. The mean urea concentration did not statistically
 229 differ from baseline (53 ± 76 mg/dL; $P > 0.05$). In group B, urea concentration increased in 5
 230 (29.4%) dogs, was equal in 2 (11.8%) and decreased in 10 (58.8%); urea was stable in 6 (35.3%) of
 231 the 17 dogs. The mean urea concentration significantly decreased by 16 mg/dL (53 ± 35 mg/dL;
 232 $P < 0.05$) (Figure 3).

233 In group A, the UPC ratio increased in 9 (31.0%) dogs and decreased in 20 (69.0%); by arbitrarily
 234 considering UPC ratio as stable if increased or decreased by $\leq 20\%$, 7 (24.1%) of the 29 dogs had
 235 stable UPC ratio. The mean UPC ratio did not differ from baseline (3.9 ± 4.9 ; $P > 0.05$). In group B,
 236 the UPC ratio increased in 2 (11.8%) dogs, was equal in 1 (5.9%) and decreased in 14 (82.3%);
 237 UPC ratio was stable in 5 (29.4%) of the 17 dogs. The mean UPC ratio significantly decreased by
 238 1.9 (2.4 ± 3.5 ; $P < 0.01$) (Figure 4).

239 **The exact time of examination performed within the 4-8 weeks interval did not differ between**
 240 **groups (5.5 ± 1.0 weeks, both groups).**

241

242 *Dogs with hypoalbuminemia*

243 In group A, AA supplementation increased serum albumin concentration at 4-8 weeks compared to
 244 baseline in all 11 dogs with severe hypoalbuminemia (serum albumin < 2.0 g/dL). The mean
 245 albumin concentration significantly increased by 0.70 g/dL ($P < 0.001$). None of these dogs had
 246 detectable subcutaneous edema or ascites, based on physical examination or abdominal
 247 ultrasonography, respectively. No significant differences were observed for BW, serum creatinine
 248 and urea concentrations, or the UPC ratio. At 4-8 weeks, the 9 dogs with hypoalbuminemia between
 249 2.0 and 2.7 g/dL had no significant change compared to baseline in BW, in serum albumin,
 250 creatinine and urea concentrations, or in UPC ratio.

251 In group B, the 4 dogs with severe hypoalbuminemia at 4-8 weeks compared to baseline had
 252 albumin concentration that was decreased in 2 of them and was equal and increased in one of each

253 remaining. Due to the limited number of cases (4 dogs), statistical analyses were not performed for
254 BW, serum albumin, creatinine and urea concentrations, or the UPC ratio. The 7 dogs with
255 hypoalbuminemia (serum albumin between 2.0 and 2.7 g/dL) had no significant change in BW,
256 serum albumin, creatinine and urea concentrations, or the UPC ratio at 4-8 weeks compared to
257 baseline.
258

259 Discussion

260 A significant increase in serum albumin concentration compared to baseline was evident in
261 proteinuric CKD dogs showing severe hypoalbuminemia (serum albumin <2.0 g/dL) when
262 receiving the AA supplementation. Along with a beneficial effect on serum albumin level,
263 supplementation with AA also increased dogs' BW, albeit mildly. The effect on BW was evident in
264 the whole group of proteinuric CKD dogs but not in those with severe hypoalbuminemia, possibly
265 due to the worse nitrogen balance of the latter cases. On the other hand, even though
266 supplementation with AA increased BW and serum albumin concentration in proteinuric CKD
267 dogs, it delayed the decrease of proteinuria and prevented lowering of urea.

268 Indeed, at follow-up, proteinuria and urea significantly decreased in dogs that did not receive AA
269 while they did not differ in dogs supplemented with AA. It is therefore possible that in these dogs
270 the reduced efficacy of the enalapril and commercial renal diet on either proteinuria or azotemia
271 was a direct consequence of the positive nitrogen balance and increased protein synthesis induced
272 by the AA supplementation. In fact, **diets lower in protein compared to maintenance diets** offer a
273 chance to reduce the overall renal trafficking of protein, and if serum protein can be lowered then
274 there is less risk of protein overload across the glomerular barrier, thus leading to less tubular
275 protein reabsorption and inflammation (3). The amount of proteins in the diet has a well-known
276 effect on the magnitude of proteinuria, and dogs fed with a **diet lower in protein compared to**
277 **maintenance diets** have reduced proteinuria, which can in turn improve serum albumin
278 concentration despite the reduction of albumin synthesis that can occur in CKD dogs (2,3,8). On the
279 other hand, because a too strict restriction of protein intake can lead to loss of BW and decreased
280 plasma albumin concentration, in proteinuric CKD dogs the protein amount administered daily with
281 food should be tailored to the degree of proteinuria; in these patients, dietary therapy should
282 minimize proteinuria and control plasma albumin concentration while not compromising the
283 nutritional status (8,24). The correct amount of proteins might differ depending on the dog's stage
284 of renal disease and entity of proteinuria (8). The commercial renal diets currently available in **dogs**

285 **have lower protein compared to maintenance diets** and it is possible they do not meet the
286 minimum requirements in case of severe proteinuria (thus leading to hypoalbuminemia and loss of
287 BW). Meanwhile, the degree of proteinuria is strictly associated with survival and CKD progression
288 in dogs (1,3,11). In light of these findings, it is the authors' opinion that supplementation with AA
289 should be carried out with caution in proteinuric CKD dogs, but it might be considered as an
290 adjunctive therapy in severely hypoalbuminemic dogs in which the anti-proteinuric treatment has
291 failed to control proteinuria and maintain plasma albumin concentration within normal limits.
292 This study has some limitations including its retrospective nature and consequent lack of blinding.
293 It is, therefore, possible that some of the effects would have been different if cases were randomly
294 allocated to receive or not the AA supplementation and the 2 groups were more homogeneous.
295 Indeed, serum concentration of creatinine at baseline was significantly higher in dogs that did not
296 receive the AA supplementation. Then, it cannot be excluded that administering AA
297 supplementation to dogs with higher creatinine concentration is associated with detrimental effects
298 on renal function. In addition, follow-up time of all dogs included in the study was short. A longer
299 follow-up period might have allowed depicting additional differences between the groups.
300 Additionally, even though owners were instructed to feed their dogs just with one of the two renal
301 diets available, sometimes they switched to the other. However, the effect of this potential bias was
302 probably minor because both commercial renal diets were expected to be **casually** provided to dogs.
303 Furthermore, studies comparing the effect of different diets in dogs with CKD have not been
304 published yet but it is likely that the two commercial renal diets used for the present study provided
305 similar beneficial effects. The IRIS simply suggests the use of a renal diet, without offering specific
306 guidance on a particular brand on the market (14).
307 Another limitation is represented by the fact that from medical records it was possible to retrieve
308 BW but not the body or the muscle condition score of the dogs; the latter might have provided more
309 information regarding the potential beneficial effect of AA supplementation . Furthermore, the
310 increase of BW in dogs receiving supplementation of AA at follow-up might have been biased by

311 the concurrent presence of subcutaneous edema or abdominal effusion; however, none of the dogs
312 with severe hypoalbuminemia in the present group developed any of the above. With regard to the
313 same group of dogs, it is worth noting that at follow-up BW increased on average by only 6.2%,
314 thus the beneficial effect of AA supplementation would be questionable. However, by considering
315 the 16 dogs that had an increase of BW, the increase was from 0.5 to 4 kg, possibly suggesting a
316 more relevant gain.

317 Furthermore, the re-evaluation at 4-8 weeks may be considered a rather large interval, which might
318 have affected the results. Although this hypothesis is conceivable, the potential bias was evenly
319 distributed in the two groups, likely limiting the source of error.

320 Another factor that might have affected the study results is that the AA provided with the
321 supplementation were predominantly essential AA. Even though it has been demonstrated that
322 people with CKD have a decrease of circulating essential AA relative to non-essential AA, (25,26)
323 there are no data available regarding the AA **blood** profile of dogs with renal disease, particularly in
324 those affected by spontaneous proteinuric CKD. Determining the AA profile of these dogs might
325 prove useful to identify the specific AA that are needed to correct their imbalance.

326 Finally, even though BW and serum albumin concentrations have been historically considered as
327 insensitive and late indicators of malnutrition, in a previous study these values were considered
328 clinically useful in assessing the adequacy of the nutritional status in dogs with renal proteinuria (8).
329 Our results support the notion that serum albumin concentration represents a helpful indicator to
330 plan dietary modification in proteinuric dogs affected by spontaneous CKD.

331 In conclusion, proteinuric CKD dogs treated with enalapril and fed commercial renal diets that
332 received supplementation with AA had improved BW and serum albumin concentration, while
333 maintaining stable serum creatinine. However, administration of AA might delay the reduction of
334 proteinuria and prevent lowering of urea. In light of these findings, the authors propose to address
335 the AA supplementation to proteinuric dogs with severe hypoalbuminemia that are not adequately
336 controlled with standard treatments consisting of renal diets and ACEI. Relying on serum albumin

337 was useful to identify the benefits of dietary changes in proteinuric dogs with CKD. Further clinical
338 trials are expected to be valuable in order to evaluate the impact of different AA formulations on
339 BW, hypoalbuminemia, and survival time of dogs affected by severe proteinuric CKD.

340

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344

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415 **Figure legends**

416 Fig. 1. Dot plot of BW in dogs in the group A and in the group B at baseline and after 4-8 weeks.

417 After 4-8 weeks, BW significantly increased in the group A whereas did not change in the group B.

418

419 Fig. 2. Dot plot of serum albumin concentration in dogs in the group A and in the group B at

420 baseline and after 4-8 weeks. After 4-8 weeks, albumin significantly increased in the group A

421 whereas did not change in the group B.

422

423 Fig. 3. Dot plot of serum urea concentration in dogs in the group A and in the group B at baseline

424 and after 4-8 weeks. After 4-8 weeks, urea significantly decreased in the group B whereas did not

425 change in the group A.

426

427 Fig. 4. Dot plot of UPC ratio in dogs in the group A and in the group B at baseline and after 4-8

428 weeks. After 4-8 weeks, UPC ratio significantly decreased in the group B whereas did not change in

429 the group A.

430

431

432 **Tables**

433 Table 1. Composition of the amino acid supplementation (100 grams).

Branched-chain aliphatic amino acids: isoleucine, leucine, valine	26 g
Aliphatic amino acids: threonine, arginine, lysine	24 g
Sulfur-containing amino acids: cysteine, methionine	7 g
Aromatic amino acids: tyrosine, phenylalanine	11 g
Heterocyclic amino acids: tryptophan, histidine	6 g
Carrier: glucose sucrose pregelatinized rice	10 g 10 g 6 g

434

435

436 Table 2. Age, BW, serum concentration of creatinine, urea and albumin, as well as UPC ratio in
 437 dogs receiving AA supplementation (group A) and in dogs not receiving AA supplementation
 438 (group B), at baseline.

439

	Group A	Group B
	(mean ± SD)	(mean ± SD)
Age (years)	6 ± 3	6 ± 3
BW (kg)	30 ± 14	28 ± 15
Creatinine (mg/dL)	2.9 ± 1.3	4.6 ± 2.8
Urea (mg/dL)	66 ± 30	69 ± 45
Albumin (g/dL)	2.4 ± 0.7	2.4 ± 0.7
UPC ratio	4.1 ± 4.5	4.3 ± 4.6

440

441 SD, standard deviation; BW, body weight; UPC, urine protein to creatinine.

442